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# Identification of Phenylphthalazinones as a New Class of *Leishmania infantum* Inhibitors

Maarten Sijm,<sup>[a]</sup> Erik de Heuvel,<sup>[a]</sup> An Matheeußen,<sup>[b]</sup> Guy Caljon,<sup>[b]</sup> Louis Maes,<sup>[b]</sup> Geert-Jan Sterk,<sup>[a]</sup> Iwan J. P. de Esch,<sup>[a]</sup> and Rob Leurs<sup>\*[a]</sup>

Leishmaniasis is a neglected parasitic disease caused by over 20 different *Leishmania* species. Current treatments often rely on harsh regimes of pentavalent antimonials such as sodium stibogluconate, while more recent drugs suffer other shortcomings such as low stability and rapid emergence of treatment failure, amongst others. Furthermore, the effectiveness of drugs varies depending on the infecting *Leishmania* species, thus there is an urgent need for new and effective anti-leishmanial drugs. Screening of an in-house compound library identified the hexahydrophthalazinone NPD-2942 as a low micromolar hit with a pIC<sub>50</sub> of 5.8 against *L. infantum* and a pIC<sub>50</sub> of 4.6 for cytotoxicity against human MRC-5 fibroblasts. To derive

structure–activity relationships, we modified the cyclohexyl ring of the hexahydrophthalazinone scaffold and 1,2,3-triazoles were attempted as replacement for the pyrazole ring, amongst others. Ultimately, the 2,3-pyrazole-substituted hexahydrophthalazinone NPD-1289 was identified as the most potent analogue in this series with a pIC<sub>50</sub> of 6.3, although some cytotoxicity toward MRC-5 cells (pIC<sub>50</sub> = 5.1) was recorded as well. Replacement of the unsubstituted 2,3-pyrazole with 1,2,3-triazoles led to compounds with lower anti-leishmanial activity. The current scaffold is a valuable new starting point for optimization toward novel anti-leishmanial drugs.

## Introduction

Leishmaniasis is a neglected parasitic disease which comes in several distinct clinical manifestations and is caused by over 20 different *Leishmania* species which are transmitted by phlebotomine sand flies.<sup>[1]</sup> Visceral leishmaniasis (VL) is caused by *L. donovani* and *L. infantum*, with 500,000 new infections annually resulting in 50,000 deaths, although this number could be higher due to underreporting.<sup>[2]</sup> Estimations are that another 1.5 million people are suffering from cutaneous leishmaniasis (CL), which is caused by almost all other *Leishmania* species.<sup>[3]</sup>

For 70 years the standard treatment of leishmaniasis is with pentavalent antimonials meglumine antimonate (1) and sodium stibogluconate (2) (Figure 1).<sup>[4,5]</sup> While quite effective outside the Indian subcontinent, the treatment regimens of 3 daily injections for 30 days, often accompanied with severe pains, are far from ideal.<sup>[3,6]</sup> Furthermore, treatment is relatively expensive and often requires additional medical supervision as a result of side effects such as vomiting, cardiotoxicity and hepatotoxicity.<sup>[7]</sup> Due to different effectiveness of antimonials against the various *Leishmania* species and emerging drug resistance, amphotericin B (3) is currently recommended as first-line treatment.<sup>[2,3,8]</sup> Some patients however suffer from

infusion reactions like chills and fever, while serious toxicity has been reported as well.<sup>[9]</sup> As such, much research has been directed toward improved formulations of amphotericin B (3) to enhance drug delivery and reduce toxicity.<sup>[9]</sup>

Due to the high cost and low stability of amphotericin B (3), pentavalent antimonials are still commonly used in rural areas where a cold chain is absent. Other newly introduced drugs also have serious concerns. The phospholipid miltefosine (4) is the first oral drug available against leishmaniasis, initially showing high cure rates of 95% with only minor side effects.<sup>[10]</sup> However, more recent reports from the clinic indicate substantial levels of treatment failure.<sup>[11–13]</sup> The aminoglycoside paromomycin (5) shows cure rates up to 85% with a treatment regime for a 35 kg person costing less than 5 euro.<sup>[14,15]</sup> The largest downside of paromomycin is the relatively long treatment regime of injections for 21 days. Experimental resistance selection also showed a high propensity for resistance development when used in monotherapy.<sup>[16]</sup> Combination treatments with sodium stibogluconate (2) improve the effectiveness for VL to cure rates of ~93%.<sup>[17]</sup>

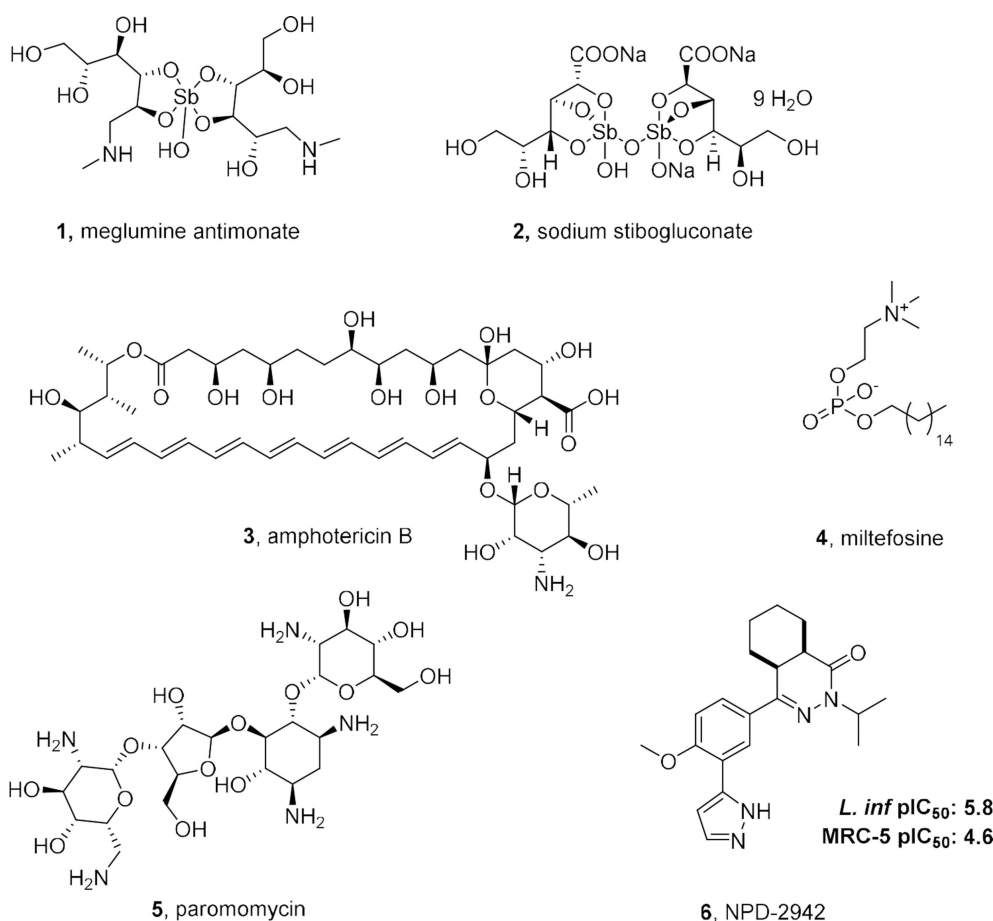
The last decades several drugs have been added to the toolbox against leishmaniasis, although each drug has its limitations.<sup>[14]</sup> Furthermore, there is a large interspecies difference of drug susceptibility and each clinical form of leishmaniasis has different requirements toward the pharmacokinetics of a drug. Hence, there remains an urgent need for new therapeutics.

To identify new hits against *Leishmania*, an in-house compound library was screened against *Leishmania infantum*. The majority of compounds in this library were previously obtained from other anti-parasitic programs. From this screening, hexahydrophthalazinone (6) was identified as a hit with a pIC<sub>50</sub> of 5.8 against *L. infantum* and low toxicity against human MRC-5 cells. To investigate the structure–activity relationships

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**Figure 1.** Pentavalent antimony drugs meglumine antimonate (1), sodium stibogluconate (2), amphotericin B (3), miltefosine (4), paromomycin (5), and identified hit NPD-2942 (6).

(SAR) for this potential new chemical class of antiparasitic drugs, the cyclohexane part of the hexahydrophthalazinone, the phthalazinone-*N*-substituent and the aromatic substituent of the anisole ring were selected for modification.

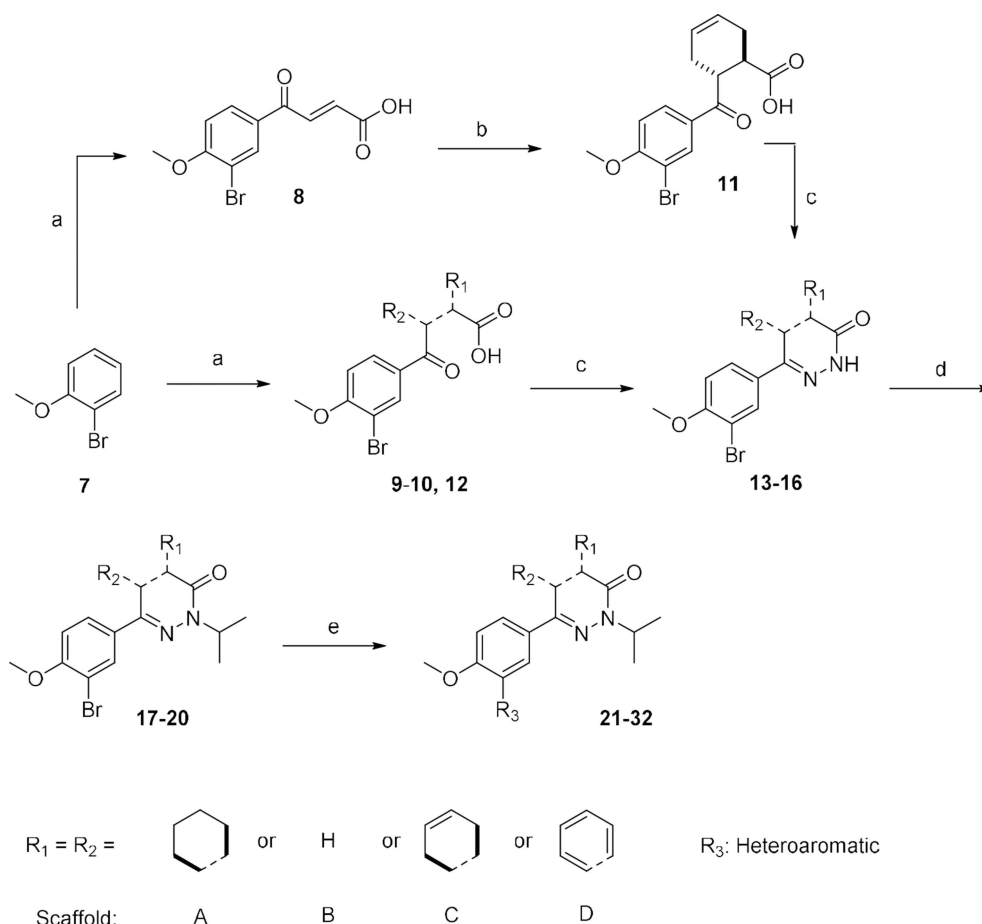
## Chemistry

The first step of the synthesis of hexahydrophthalazinone analogues was the Friedel-Crafts acylation of 2-bromoanisole with several anhydrides, yielding the corresponding keto-acids (**9–10**, **12**, Scheme 1). The keto-acid precursor (**11**) for tetrahydrophthalazinone was prepared in two steps; first a Friedel-Crafts acylation was done with maleic anhydride leading to trans-keto-acid **8**, which was followed by a Diels-Alder reaction with 1,3-butadiene resulting in trans-keto-acid **11**. Ring-closure of these keto-acids with hydrazine yielded the desired hexahydrophthalazinone (**13**), dihydropyridazinone (**14**), tetrahydrophthalazinone (**15**) and phthalazinone (**16**). An isopropyl moiety was installed on the unsubstituted phthalazinones and pyridazine using sodium hydride and isopropylbromide (**17–20**). In case of tetrahydrophthalazinone (**11**) the alkylation reaction with NaH resulted in the conversion of the trans-isomer to the cis-isomer as previously

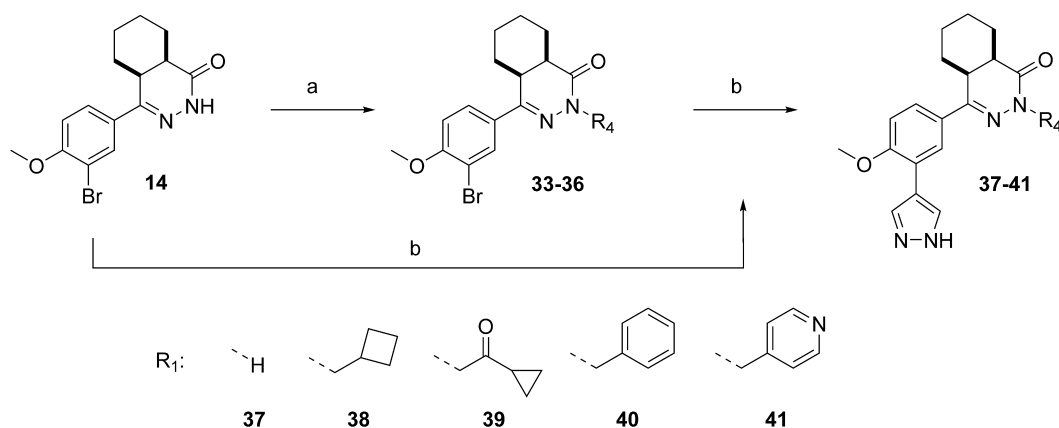
reported by de Heuvel *et al.*<sup>[15]</sup> After the alkylation the final aromatic substituents could be introduced via a Suzuki cross-coupling resulting in analogues substituted with various pyrazoles (**21–23**, **26–32**), 2-furan (**24**) and 3-thiophene (**25**).

Further differentiation of the substituents on the hexahydrophthalazinone nitrogen (Scheme 2), was done by deprotonation with sodium hydride followed by addition of the desired alkyl halides (**33–36**). On the alkylated intermediates the so far most promising 4-*H*-pyrazolone was installed using a Suzuki cross-coupling (**37–41**).

Introduction of the 1,2,3-triazoles was done starting with isopropyl hexahydrophthalazinone **18** (Scheme 3), which was used in a Sonogashira reaction with trimethylsilylacetylene to yield **42**. The TMS protecting group (**42**) was removed with potassium hydroxide in MeOH, resulting in the acetylene substituted building-block (**43**). The desired azides were prepared *in situ* by refluxing sodium azide with the desired alkyl halide in MeOH/H<sub>2</sub>O (8:1).<sup>[16]</sup> After 16 hours this mixture was cooled to room temperature and a portion of the azide solution was added to a mixture of the acetylene building block (**43**), CuSO<sub>4</sub> and L-ascorbate, yielding the desired 1,2,3-triazoles (**44–51**) after stirring overnight at room temperature.



**Scheme 1.** Synthesis of various phthalazinones and pyridazinones: a) AlCl<sub>3</sub>, anhydride, CH<sub>2</sub>Cl<sub>2</sub>, RT, 16 h, 58–78 %, b) buta-1,3-diene, THF, 140 °C, 30 min, 10 bar, 97 %. c) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O, EtOH, RT–60 °C, 16 h, 20–89 %, d) isopropyl bromide, NaH, DMF, 50 °C, RT, 81–90 %, e) R<sub>3</sub>-B(OH)<sub>2</sub> or R<sub>3</sub>-pinacol, Na<sub>2</sub>CO<sub>3</sub>, Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O/DME (1:3), 120 °C, 1 h, 8–26 %.

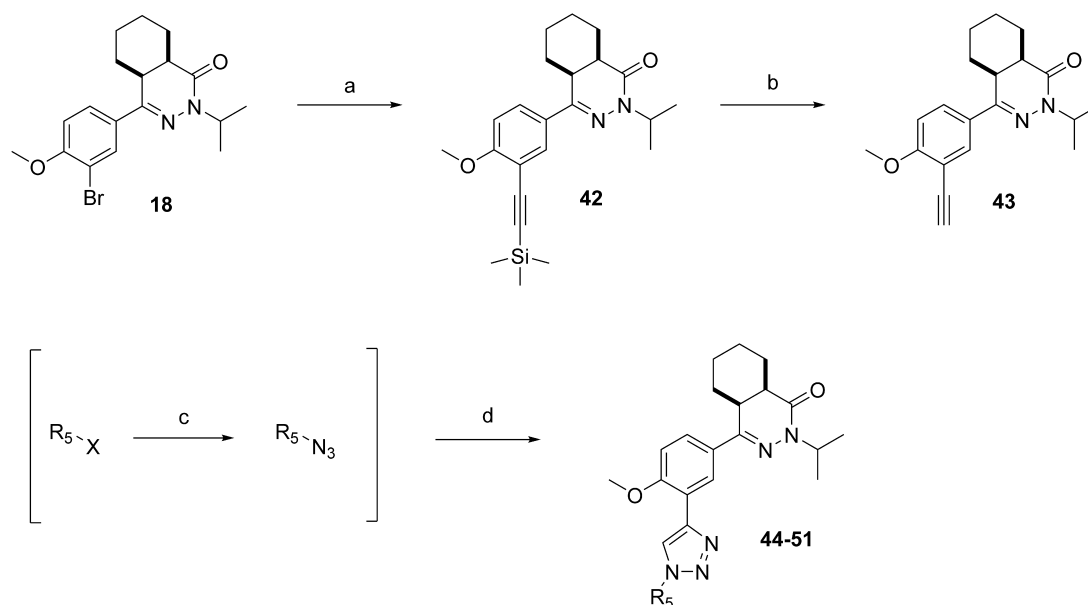


**Scheme 2.** Synthesis of hexahydrophthalazinones: a) alkyl bromide or chloride, NaH, DMF, 50 °C, RT, 57–92 %, b) pyrazole-B(OH)<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O/DME (1:3), 120 °C, 1 h, 14–29 %.

## Results and Discussion

The first series of analogues synthesized around hit molecule NPD-2942 (**6**, Figure 1) had modifications at two distinct

positions. By modifying R<sub>1</sub> and R<sub>2</sub>, the role of the hexyl ring of the hexahydrophthalazinone scaffold was investigated, while modifications on R<sub>3</sub> were introduced to investigate the SAR of the hetero-aromatic substituent (Table 1). Introduction of a



**Scheme 3.** Synthesis of triazole substituted hexahydrophthalazinones: a)  $\text{Pd(PPh}_3)_4$ , TEA, TMS-acetylene, 100 °C, 1 h, 93 %, b) KOH, MeOH, RT, 86 %, 16 h, c)  $\text{NaN}_3$ , MeOH/H<sub>2</sub>O (8:1), 80 °C, 16 h, d)  $\text{R}_1\text{-N}_3$ ,  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ , L-ascorbate, MeOH/H<sub>2</sub>O (8:1), RT, 16 h, 34–59 %.

methyl group on the 1,2-pyrazole (**21**) reduced antiparasitic activity to 5.1 ( $\text{pIC}_{50}$ ). Instead, installation of a 2,3-pyrazole ring, either unsubstituted (**22**) or methylated (**23**), improved activity compared to the original hit (**6**), with both compounds showing  $\text{pIC}_{50}$  values of 6.3. Cytotoxicity toward human MRC-5 cells was however higher for the methylated analogue (**23**,  $\text{pIC}_{50}$  = 5.6, SI = 5) than the unsubstituted pyrazole (**22**,  $\text{pIC}_{50}$  = 5.1, SI = 16). Introduction of other heterocyclic rings, such as a 2-furan (**24**) and 3-thiophene ring (**25**) resulted in compounds with decreased activities ( $\text{pIC}_{50}$  values around 5.1). The removal of the cyclohexene ring (**26–27**) resulted in  $\text{pIC}_{50}$  values of 4.5, which is a decrease of 1.3 log units compared to original hit NPD-2942 (**6**). This shows the importance of having a cyclohexane ring on this position. Introduction of a cyclohexene ring instead of a cyclohexane, as in compounds **28–30**, resulted in a small increase in activity compared to the original hit (**6**) for the unsubstituted 1,2-pyrazole (**28**) with a  $\text{pIC}_{50}$  of 6.0. However, MRC-5 cytotoxicity of **28** also increased with 0.3 log unit showing 13 fold selectivity toward *L. inf.* The unsubstituted 2,3-pyrazole (**29**) had similar potency as the original hit (**6**) ( $\text{pIC}_{50}$  = 5.7) while the methylated analogue (**30**), was more potent with a  $\text{pIC}_{50}$  of 6.3. This increased potency of **30** was however accompanied with an increase of MRC-5 toxicity ( $\text{pIC}_{50}$  = 5.4). Replacing the cyclohexane ring with a phenyl moiety is tolerated, as 2,3-pyrazoles **31** and **32** showed  $\text{pIC}_{50}$  values of 5.6 and 6.0 respectively for the unsubstituted and methylated analogues. Both compounds, however, also showed some MRC-5 cytotoxicity with  $\text{pIC}_{50}$  values around 5.0.

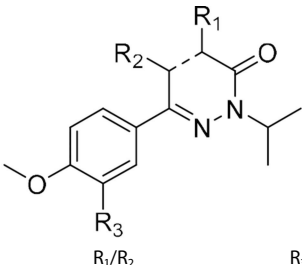




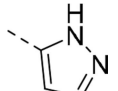
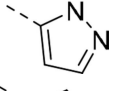
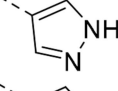
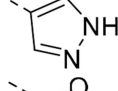
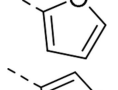
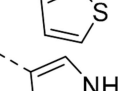
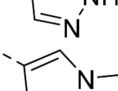
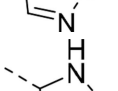
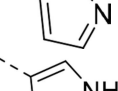
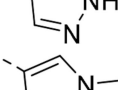
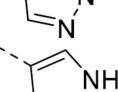
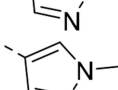
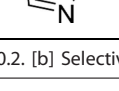
After this first round of SAR, it was decided to continue with the unsubstituted 2,3-pyrazole on the  $\text{R}_3$  position and a cyclohexene ring on  $\text{R}_1$  and  $\text{R}_2$ . This compound, NPD-1289 (**22**), which has 16 fold selectivity over human cells with a  $\text{pIC}_{50}$  of 6.3 and a MRC-5 cytotoxicity of 5.1 ( $\text{pIC}_{50}$ ), showed the best

selectivity index in this series. The next position which was investigated for optimization was the substituent of the phthalazinone nitrogen ( $\text{R}_4$ , Table 2).

Removal of the isopropyl moiety led to compound **37**, which was considered inactive ( $\text{pIC}_{50}$  = 4.2) against *L. infantum* but did show substantial cytotoxicity toward MRC-5 cells ( $\text{pIC}_{50}$  = 5.5). Installation of a cyclobutylmethyl instead of the isopropyl moiety of **22** resulted in analogue **38**. This molecule shows low micromolar activity against *L. infantum* ( $\text{pIC}_{50}$  = 5.9), but is accompanied with substantial cytotoxicity ( $\text{pIC}_{50}$  = 5.6). The three cyclopropyl-2-oxoethyl **39**, benzyl **40** and 4-pyridinylmethyl **41** analogues all showed even higher cytotoxicity for MRC-5 cells compared to their activities against *L. infantum*. Based on these findings, further modifications of the  $\text{R}_4$  position were discontinued for now.

Next, analogues were prepared with modifications on the heteroaromatic ring on  $\text{R}_5$  (Table 3), aiming for lower toxicity. Moving away from the pyrazoles, instead 1,2,3-triazoles were introduced by utilizing click reactions on acetylene substituted hexahydrophthalazinone **43**. Having small aliphatic substituents on the 1,2,3-triazole (**44–47**) resulted in four compounds with activities around 5.7 ( $\text{pIC}_{50}$ ), however large differences in MRC-5 toxicity were observed. While methylcyclopropyl **44** and butyronitrile (**47**) showed some cytotoxicity against MRC-5 cells ( $\text{pIC}_{50}$  = 5.0 and 4.7), methylcyclobutyl (**45**), cyclopentyl (**46**) showed virtually no cytotoxicity with  $\text{pIC}_{50}$  values of 4.3 and <4.2 respectively, resulting in high SI's of 25 and >31. Attempts to introduce some polar side chains, by installing morpholine moieties (**48**, **49**), resulted in compounds with moderate activities against *L. infantum* with  $\text{pIC}_{50}$  values of 5.1 and 4.7 respectively, while showing cytotoxicity toward MRC-5 cells at similar concentrations. Installing aromatic moieties, such as benzyl derivatives **50** and **51**, resulted in two compounds

**Table 1.** Phenotypic activity of various *N*-isopropyl phthalazinones and dihydropyridazinone against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cell growth.






Compd					
		A	B	C	D
			pIC <sub>50</sub> <sup>[a]</sup> <i>L. inf</i>	MRC-5	SI <sup>[b]</sup>
6 NPD-2942	A		5.8	4.6	16
21	A		5.1	4.6	3
22	A		6.3	5.1	16
23	A		6.3	5.6	5
24	A		5.1	4.5	4
25	A		5.2	< 4.2	> 10
26	B		4.5	4.5	1
27	B		4.5	4.5	1
28	C		6.0	4.9	13
29	C		5.7	4.6	13
30	C		6.3	5.4	8
31	D		5.6	5.2	3
32	D		6.0	5.0	10

[a] All reported values are within a standard deviation of  $\pm 0.2$ . [b] Selectivity index:  $IC_{50}(L. inf)/IC_{50}(MRC-5)$ .

with activities around 5.4 (pIC<sub>50</sub>) and no observable toxicity against MRC-5 cells (pIC<sub>50</sub> < 4.2). While 1,2,3-triazoles showed substantially lower cytotoxicity than their pyrazole counterparts, their antiparasitic activities are also lower. The cyclopentyl

substituted triazole, NPD-3189 (**46**) does however show some promise with a pIC<sub>50</sub> of 5.7 and no toxicity.

**Table 2.** Phenotypic activity of hexahydrophthalazinones with various N-substituents against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cells.


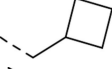
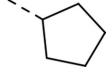
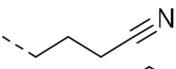
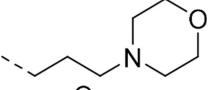
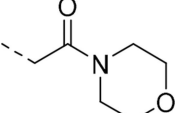
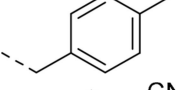
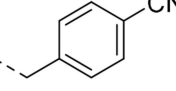
Compd	R <sub>4</sub>	pIC <sub>50</sub> <sup>[a]</sup> <i>L. inf</i>	MRC-5	SI <sup>[b]</sup>
37		4.2	5.5	0.05
38		5.9	5.6	2
39		4.4	5.0	0.25
40		5.4	5.6	0.63
41		5.4	6.1	0.20

[a] All reported values are within a standard deviation of  $\pm 0.2$ . [b] Selectivity index: IC<sub>50</sub>(*L. inf*)/IC<sub>50</sub>(MRC-5).

## Conclusions

Starting from the screening hit NPD-2942 (**6**) a series of close analogues has been prepared to investigate SAR around the hexahydrophthalazinone scaffold. Variations of the core scaffold shows that removal of the cyclohexane moiety leads to compounds with decreased activity (pIC<sub>50</sub>s around 4.5), modification of the cyclohexane to a cyclohexene or phenyl moiety is tolerated, although this generally does not improve potency. Introduction of several heteroaromatic rings on R<sub>3</sub> revealed that an unsubstituted 2,3-pyrazole on the hexahydrophthalazinone scaffold (**22**, NPD-1289) is the most promising analogue of this series with a pIC<sub>50</sub> value of 6.3 and a 16-fold selectivity over toxicity for MRC-5 cells. Close analogue **30** shows a similar potency, however is less promising with a relative high cytotoxicity (pIC<sub>50</sub> value=5.4, SI=8). Modifications of the isopropyl moiety (**37–41**) led to compounds with high cytotoxicity toward MRC-5 cells and this position was not further investigated. Replacing the unsubstituted 2,3-pyrazole with 1,2,3-triazoles (**44–51**) yielded a set of compounds with lower activity against *L. infantum*, although higher selectivity over MRC-5 cells was achieved with methylcyclobutyl **45** (SI=25) and cyclopentyl **46** (SI>31). Overall, the current series shows the promise of the hexahydrophthalazinone scaffold. This new chemical class shows sub-micromolar anti-leishmanial activity

**Table 3.** Phenotypic activity against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cell growth by 1,2,3-triazole substituted hexahydrophthalazinones.

Compd	R <sub>5</sub>	pIC <sub>50</sub> <sup>[a]</sup> <i>L. inf</i>	MRC-5	SI <sup>[b]</sup>
44		5.7	5.0	5
45		5.7	4.3	25
46		5.7	< 4.2	> 31
47		5.6	4.7	8
48		5.1	4.7	3
49		4.5	4.5	1
50		5.2	< 4.2	> 10
51		5.5	< 4.2	> 20

[a] All reported values are within a standard deviation of  $\pm 0.2$ . [b] Selectivity index: IC<sub>50</sub>(*L. inf*)/IC<sub>50</sub>(MRC-5).

and is a valuable starting point for further anti-leishmanial hit-to-lead optimization.

## Experimental Section

### Biology

**Leishmania infantum cellular assay:** *L. infantum* MHOM/MA(BE)/67 amastigotes were collected from the spleen of an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine *in vitro* anti-leishmanial activity,  $3 \times 10^4$  macrophages were seeded in each well of a 96-well plate. After 2 d of outgrowth,  $5 \times 10^5$  amastigotes/well were added and incubated for 2 h at 37 °C. Solutions with or without test compound were subsequently added, and the plates were further incubated for 5 d at 37 °C and



5% CO<sub>2</sub>. Parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed on 500 cells after Giemsa staining of the test plates and expressed as a percentage of the blank controls without test compound. In the case of observed toxicity to the macrophages, the lowest concentration was recorded at which the toxicity was observed, and this was used as a qualitative phenotypic assessment.<sup>[17]</sup> As a positive control Miltefosine was used.

**MRC-5 cytotoxicity cellular assay:** MRC-5 SV2 cells, originally from a human diploid lung cell line, were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCS. For the assay, 10<sup>4</sup> cells/well were seeded onto the test plates containing the pre-diluted sample and incubated at 37 °C and 5% CO<sub>2</sub> for 72 h. Cell viability was assessed fluorimetrically 4 h after addition of 10 µg/mL resazurin (excitation 550 nm, emission 590 nm). The results are expressed as percentage reduction in cell viability compared to untreated controls. Tamoxifen was used as a positive control in this experiment.

## Chemistry

Chemicals and reagents were obtained from commercial suppliers and were used without further purification. Anhydrous DMF, THF and CH<sub>2</sub>Cl<sub>2</sub> were obtained by passing them through an activated alumina column prior to use. Microwave reactions were executed using a Biotage® Initiator microwave system. <sup>1</sup>H NMR spectra were recorded on a Bruker Avance 250 (250 MHz), Bruker Avance 400 (400 MHz), Bruker Avance 500 (500 MHz) or Bruker 600 Avance (600 MHz) spectrometer. Data are reported as follows: chemical shift, integration, multiplicity (s=singlet, d=doublet, dd=double doublet, t=triplet, dt=double triplet, q=quartet, p=pentet, h=heptet, bs=broad singlet, m=multiplet), and coupling constants (Hz). Chemical shifts are reported in ppm with the natural abundance of deuterium in the solvent as the internal reference (CDCl<sub>3</sub>: δ 7.26, (CD<sub>3</sub>)<sub>2</sub>SO: δ 2.50). <sup>13</sup>C NMR spectra were recorded on a Bruker Avance 500 (126 MHz) or Bruker Avance 600 (150 MHz). Chemical shifts are reported in ppm with the solvent resonance resulting from incomplete deuteration as the internal reference (CDCl<sub>3</sub>: δ 77.16 or (CD<sub>3</sub>)<sub>2</sub>SO: δ 39.52). Systematic names for molecules according to IUPAC rules were generated using the Chemdraw AutoName program. LC–MS data was gathered using a Shimadzu HPLC/MS workstation with a LC-20AD pump system, SPD–M20 A diode array detection, and a LCMS-2010 EV mass spectrometer. The column used is an Xbridge C<sub>18</sub> 5 µm column (100 mm × 4.6 mm). Solvents used were the following: solvent B=MeCN, 0.1% formic acid; solvent A=water, 0.1% formic acid. The analysis was conducted using a flow rate of 1.0 mL/min, start 5% B, linear gradient to 90% B in 4.5 min, then 1.5 min at 90% B, linear gradient to 5% B in 0.5 min and then 1.5 min at 5% B, total run time of 8 min. All reported compounds have purities > 95%, measured at 254 nm, unless otherwise mentioned. All HRMS spectra were recorded on a Bruker microTOF mass spectrometer using ESI in positive-ion mode. Column purifications were either carried out automatically using Biotage equipment or manually, using 60–200 mesh silica. TLC analyses were performed with Merck F254 alumina silica plates using UV visualization. All reactions were done under N<sub>2</sub> atmosphere, unless specifically mentioned.

## Syntheses

**(E)-4-(3-Bromo-4-methoxyphenyl)-4-oxobut-2-enoic acid (8):** To an ice-cooled mixture of 1-bromo-2-methoxybenzene (**6**) (65 mL, 0.52 mol) and furan-2,5-dione (77 g, 0.78 mol) in CH<sub>2</sub>Cl<sub>2</sub> (465 mL) was added AlCl<sub>3</sub> (84 g, 0.63 mol). The reaction mixture was stirred at RT overnight. The orange suspension was quenched in 3 M aq.

HCl (1.5 L) and extracted using EtOAc (4 × 1 L). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain a dark yellow solid. Trituration with Et<sub>2</sub>O provided the product as a light yellow solid (97.5 g, 66%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.89 (d, *J* = 2.2 Hz, 1H), 7.69 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.53 (d, *J* = 15.4 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.51 (d, *J* = 15.4 Hz, 1H), 3.69 (s, 3H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>): δ 187.2, 167.1, 166.7, 160.2, 133.8, 133.0, 131.3, 130.6, 112.9, 111.8, 57.3. LC–MS (ESI) *m/z* found: 285 [M + H]<sup>+</sup>; retention time: 4.09 minutes. HRMS (ESI): *m/z*: [M + H]<sup>+</sup> calcd. for C<sub>11</sub>H<sub>10</sub>BrO<sub>4</sub> 284.9757, found 284.9763.

**(1R,2S)-2-(3-Bromo-4-methoxybenzoyl)cyclohexanecarboxylic acid (9):** *cis*-hexahydroisobenzofuran-1,3-dione (17.3 g, 112 mmol) was added to a round bottom flask, followed by 1-bromo-2-methoxybenzene (**6**) (20 g, 107 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (250 mL). The mixture was stirred and cooled with an ice-bath. Aluminum trichloride (17.1 g, 128 mmol) was added in small portions over approximately 5 min, after which the ice-bath was removed and the mixture stirred for 16 h, by then the mixture was a deep red solution. The mixture was quenched in a mixture of ice and concentrated hydrochloric acid (3:1) after which a white solid precipitated. Solids were filtered off, washed with water (3 × 50 mL) and dried *in vacuo* to yield 24 g (63 mmol, 58%) of the title compound. <sup>1</sup>H-NMR: (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.05 (s, 1H), 8.04 (d, 8.6 Hz, 1H), 7.94 (d, 8.6 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 3.93 (s, 3H), 3.39 (s, 1H), 2.73–2.58 (m, 1H), 2.03 (q, *J* = 9.5 Hz, 1H), 1.93–1.78 (m, 2H), 1.78–1.67 (m, 1H), 1.67–1.56 (m, 1H), 1.44–1.23 (m, 2H), 1.24–1.10 (m, 1H). LC–MS (ESI) *m/z* found: 341 [M + H]<sup>+</sup>; retention time: 4.43 minutes, purity: 85%. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>15</sub>H<sub>18</sub>BrO<sub>4</sub>: 341.0383, found 341.0392.

**trans-6-(3-Bromo-4-methoxybenzoyl)cyclohex-3-ene-1-carboxylic acid (11):** A mixture of keto-acid **8** (70.0 g, 221 mmol) and buta-1,3-diene in THF (~13% w/w, 150 mL, 300 mmol) was divided over 8 microwave vials. Each vial was stirred under microwave irradiation at 140 °C for 30 min (the internal pressure reached ~10 bar). The reaction mixtures were pooled and concentrated *in vacuo* to obtain the crude product. Trituration with toluene provided the product as a white solid (72.4 g, 97%). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): δ 12.30 (s, 1H), 8.16 (s, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 5.79–5.66 (m, 2H), 3.95 (s, 3H), 3.83–3.72 (m, 1H), 2.92–2.75 (m, 1H), 2.54–2.10 (m, 3H), 1.93–1.79 (m, 1H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>): δ 200.8, 176.4, 159.61, 133.4, 130.6, 130.3, 125.8, 125.7, 112.8, 111.6, 57.2, 42.2, 41.6, 29.4, 28.4. LC–MS (ESI) *m/z* found: 339 [M + H]<sup>+</sup>; retention time: 4.53 minutes. HRMS (ESI): *m/z*: [M + H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>16</sub>BrO<sub>4</sub> 339.0226, found 339.0213.

**(4aS,8aR)-4-(3-Bromo-4-methoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (13):** Keto-acid **10** (13.0 g, 38.1 mmol) was charged to a round bottom flask followed by ethanol (150 mL). This suspension was stirred and hydrazine hydrate (50–60% (20 mL, 381 mmol) was added in portions. The mixture was heated at 60 °C for 16 h after which it was allowed the cool to RT while stirring. White precipitation was visible and 50 mL of water was added, increasing precipitation. Solids were filtered off and dried *in vacuo* to yield 11.4 gram (33.8 mmol, 89%) of the title compound as a white solid. <sup>1</sup>H-NMR: (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.89 (s, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.76 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 3.29–3.20 (m, 1H), 2.71 (s, 1H), 2.28 (d, *J* = 12.5 Hz, 1H), 1.67 (d, *J* = 12.6 Hz, 1H), 1.56–1.47 (m, 2H), 1.45–1.33 (m, 2H), 1.30–1.16 (m, 2H). LC–MS (ESI) *m/z* found: 337 [M + H]<sup>+</sup>; retention time: 4.41 minutes. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>15</sub>H<sub>18</sub>BrN<sub>2</sub>O<sub>2</sub>: 337.0546, found 337.0536.

**(4aS,8aR)-4-(3-Bromo-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (17):** Phthalazinone **13** (5.0 g, 14.8 mmol) was dissolved in DMF (100 mL) and sodium hydride (60% in mineral oil) (652 mg, 16.3 mmol) was added. The



mixture was stirred for 15 min after which 2-bromopropane (1.67 mL, 17.8 mmol) was added and the mixture was stirred for 16 h at 60 °C. The reaction was quenched pouring the mixture in water (250 mL) and the aqueous layer was extracted with MTBE (250 mL). The organic layer was washed with sat. aqueous NaHCO<sub>3</sub> (2x 250 mL) and brine (250 mL) after which the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. The solids were filtered off and volatiles were evaporated *in vacuo* after which the remaining crude was purified over SiO<sub>2</sub> using a gradient of 10% EtOAc in heptane toward 50% EtOAc in heptane yielding 4.6 g (12.1 mmol, 82%) of the title compound as a white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 7.98 (s, 1H), 7.69 (d, *J* = 8.6 Hz, 1H), 6.90 (d, 1H), 5.03 (hept, *J* = 6.4 Hz, 1H), 3.03–2.93 (m, 1H), 2.63 (s, 1H), 2.60–2.47 (m, 1H), 1.84–1.72 (m, 1H), 1.68–1.56 (m, 2H), 1.40–1.30 (m, 3H), 1.29 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.7 Hz, 3H). <sup>13</sup>C-NMR: (126 MHz, CDCl<sub>3</sub>) δ 166.45, 156.68, 151.24, 130.75, 129.19, 126.15, 112.06, 111.47, 56.36, 46.40, 36.69, 35.48, 26.90, 25.67, 24.28, 23.98, 20.65, 20.18. LC–MS (ESI) *m/z* found: 379 [M + H]<sup>+</sup>; retention time: 5.51 minutes. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>18</sub>H<sub>24</sub>BrN<sub>2</sub>O<sub>2</sub>: 379.1016, found 379.1009.

**2-Isopropyl-4-(4-methoxy-3-(1H-pyrazol-4-yl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (22):** Phthalazinone 17 (100 mg, 0.26 mmol) and (1H-pyrazol-4-yl)boronic acid (35 mg, 0.32 mmol) were added to a microwave tube with a stirring bean, subsequently DME (3 mL) and 1 M sodium carbonate (0.9 mL, 0.9 mmol) were added and the mixture was degassed for 5 minutes with N<sub>2</sub>. After addition of PdCl<sub>2</sub>(dppf).CH<sub>2</sub>Cl<sub>2</sub> (20 mg, 0.026 mmol) the mixture was degassed for another 2 min and the vessel was sealed and heated for 1 h at 120 °C. The mixture was diluted with EtOAc (25 mL), filtered over Celite and the organic layer was washed with water (2x 25 mL) and brine (25 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, solids were filtered off and the residue was concentrated *in vacuo*. Remaining crude was purified over SiO<sub>2</sub> using a gradient of 50% heptanes in EtOAc toward 5% MeOH in EtOAc to yield the 18 mg (0.05 mmol, 19%) of title compound as an off-white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 8.08 (s, 2H), 8.04 (d, *J* = 2.3 Hz, 1H), 7.64 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 1H), 5.08 (hept, *J* = 6.6 Hz, 1H), 3.95 (s, 3H), 3.13–3.03 (m, 1H), 2.72–2.65 (m, 1H), 2.61–2.52 (m, 1H), 1.85–1.76 (m, 1H), 1.73–1.60 (m, 2H), 1.49–1.35 (m, 4H), 1.33 (d, *J* = 6.6 Hz, 3H), 1.25 (d, *J* = 6.6 Hz, 3H). <sup>13</sup>C-NMR: (126 MHz, CDCl<sub>3</sub>) δ 166.60, 156.95, 152.67, 133.16, 127.93, 125.30, 125.22, 121.55, 117.70, 110.93, 55.65, 46.40, 36.76, 35.61, 25.76, 24.44, 24.06, 22.06, 20.69, 20.24. LC–MS (ESI) *m/z* found: 367 [M + H]<sup>+</sup>; retention time: 4.55 minutes. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>21</sub>H<sub>27</sub>N<sub>4</sub>O<sub>2</sub>: 367.2129, found 367.2116.

**(4aS,8aR)-4-(3-Bromo-4-methoxyphenyl)-2-(cyclobutylmethyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (33):** Phthalazinone 13 (450 mg, 1.33 mmol) was dissolved in DMF (10 mL) and sodium hydride (60% in mineral oil) (59 mg, 1.5 mmol) was added. The mixture was stirred for 15 min after which (bromomethyl)cyclobutane (0.18 mL, 1.6 mmol) was added and the mixture was stirred for another 16 h. The reaction was quenched pouring the mixture in water (50 mL) and the aqueous layer was extracted with MTBE (50 mL). The organic layer was washed with sat. aqueous NaHCO<sub>3</sub> (2x 50 mL) and brine (50 mL) after which the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. The solids were filtered off and volatiles were evaporated *in vacuo* after which the remaining crude was purified over SiO<sub>2</sub> using a gradient of 10% EtOAc in heptane toward 70% EtOAc in heptane yielding 430 mg (1.06 mmol, 79%) of the title compound as a white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 7.97 (d, *J* = 2.1 Hz, 1H), 7.66 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 4.13 (dd, *J* = 13.2, 7.7 Hz, 1H), 3.93 (s, 3H), 3.65 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.05–2.95 (m, 1H), 2.75 (h, *J* = 7.8 Hz, 1H), 2.70–2.64 (m, 1H), 2.59–2.49 (m, 1H), 2.07–1.97 (m, 2H), 1.93–1.76 (m, 5H), 1.69–1.59 (m, 3H), 1.45–1.23 (m, 5H). <sup>13</sup>C-NMR: (126 MHz, CDCl<sub>3</sub>) δ 167.17, 156.74, 151.27, 130.83, 128.89, 126.20, 112.11, 111.47, 56.37, 53.30, 36.40,

35.96, 26.12, 26.09, 25.71, 24.40, 23.95, 22.00, 18.46. LC–MS (ESI) *m/z* found: 405 [M + H]<sup>+</sup>; retention time: 5.91 minutes. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>20</sub>H<sub>26</sub>BrN<sub>2</sub>O<sub>2</sub>: 405.1172, found 405.1165.

**(4aS,8aR)-2-(Cyclobutylmethyl)-4-(4-methoxy-3-(1H-pyrazol-4-yl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (38):** Phthalazinone 33 (150 mg, 0.37 mmol) and (1H-pyrazol-4-yl)boronic acid (62 mg, 0.56 mmol) were added to a microwave tube with a stirring bean, subsequently DME (4 mL) and 1 M sodium carbonate (2 mL, 2.0 mmol) were added and the mixture was degassed for 5 min with N<sub>2</sub>. After addition of PdCl<sub>2</sub>(dppf).CH<sub>2</sub>Cl<sub>2</sub> (30 mg, 0.037 mmol) the mixture was degassed for another 2 minutes and the vessel was sealed and heated for 1 h at 120 °C. The mixture was diluted with EtOAc (25 mL), filtered over Celite and the organic layer was washed with water (2x 25 mL) and brine (25 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, solids were filtered off and the residue was concentrated *in vacuo*. Remaining crude was purified over SiO<sub>2</sub> using a gradient of 50% heptanes in EtOAc toward 5% MeOH in EtOAc to yield 37 mg (0.09 mmol, 25%) of the title compound as an off-white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 8.09 (s, 2H), 8.00 (d, *J* = 1.8 Hz, 1H), 7.59 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 4.17 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.94 (s, 3H), 3.68 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.14–3.03 (m, 1H), 2.82–2.68 (m, 2H), 2.61–2.51 (m, 1H), 2.12–1.97 (m, 2H), 1.94–1.76 (m, 5H), 1.73–1.59 (m, 2H), 1.50–1.29 (m, 4H). <sup>13</sup>C-NMR: (126 MHz, CDCl<sub>3</sub>) δ 167.35, 157.02, 152.87, 133.38, 127.59, 125.35, 125.22, 121.77, 117.58, 110.94, 105.69, 55.63, 53.25, 36.47, 36.12, 34.86, 26.20, 25.78, 24.55, 24.02, 22.08, 18.52. LC–MS (ESI) *m/z* found: 393 [M + H]<sup>+</sup>; retention time: 4.83 minutes. HRMS-ESI [M + H]<sup>+</sup> calculated for C<sub>23</sub>H<sub>28</sub>N<sub>4</sub>O<sub>2</sub>: 393.2285, found 393.2276.

**(4aS,8aR)-2-Isopropyl-4-(4-methoxy-3-((trimethylsilyl)ethynyl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (42):** Phthalazinone 13 (2.0 g, 5.3 mmol) was added to a microwave tube and TEA (12 mL) was added. The mixture was degassed with N<sub>2</sub> for 5 min after which Tetrakis (0.61 g, 0.53 mmol) and CuI (0.1 g, 0.53 mmol) were added. The tube was heated in the microwave at 100 °C for 1 h, after which the mixture was allowed to cool to RT, diluted with MTBE (25 mL) and filtered over Celite. The organic layer was washed with saturated aqueous NaHCO<sub>3</sub> (2x 25 mL) and brine (25 mL), after which it was dried over Na<sub>2</sub>SO<sub>4</sub> and solids were filtered off. The remaining black crude was concentrated *in vacuo* and purified over SiO<sub>2</sub> using a gradient of heptane toward 25% EtOAc in heptane yielding 1.95 g (4.9 mmol, 93%) of the title compound as a white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 7.83–7.76 (m, 2H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.05 (hept, *J* = 6.6 Hz, 1H), 3.92 (s, 3H), 3.07–2.99 (m, 1H), 2.63 (s, 1H), 2.55 (d, *J* = 9.8 Hz, 1H), 1.83–1.75 (m, 1H), 1.68–1.60 (m, 2H), 1.58 (s, 2H), 1.41–1.32 (m, 2H), 1.31 (d, *J* = 6.6 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 0.29 (s, 8H). LC–MS (ESI) *m/z* found: 397 [M + H]<sup>+</sup>; retention time: 6.09 minutes.

**(4aS,8aR)-4-(3-Ethynyl-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (43):** Phthalazinone 42 (2.0 g, 5.0 mmol) was charged to a round bottom flask, followed by MeOH (50 mL) and 2.5 M aqueous potassium hydroxide. The mixture was stirred overnight at RT for 16 h, after which the aqueous layer was extracted with EtOAc (50 mL). The organic layer was washed with brine (50 mL) and dried over Na<sub>2</sub>SO<sub>4</sub> after which the solids were filtered off. Volatiles were removed *in vacuo*, after which the remaining crude was purified over SiO<sub>2</sub> using a gradient of 10% EtOAc in heptanes toward 70% EtOAc in heptanes yielding 1.4 g (5.0 mmol, 86%) of the title compound as a white solid. <sup>1</sup>H-NMR: (500 MHz, CDCl<sub>3</sub>) δ 7.87 (d, *J* = 2.3 Hz, 1H), 7.81 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 5.05 (hept, *J* = 6.6 Hz, 1H), 3.95 (s, 3H), 3.35 (s, 1H), 3.07–2.97 (m, 1H), 2.67–2.62 (m, 1H), 2.59–2.50 (m, 1H), 1.84–1.76 (m, 1H), 1.68–1.60 (m, 2H), 1.47–1.32 (m, 4H), 1.30 (d, *J* = 6.6 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H). <sup>13</sup>C-NMR: (126 MHz, CDCl<sub>3</sub>) δ 166.47, 161.36, 151.60, 131.59, 128.01, 127.84, 111.30, 110.66, 81.51, 79.74, 56.11, 46.36, 36.70, 35.47, 25.69, 24.28, 24.01, 22.00, 20.65,

20.18. LC–MS (ESI)  $m/z$  found: 325  $[M+H]^+$ ; retention time: 5.12 minutes. HRMS-ESI  $[M+H]^+$  calculated for  $C_{20}H_{25}N_2O_2$ : 325.1911, found 325.1904.

(4a*S*,8a*R*)-4-(3-(1-(cyclopropylmethyl)-1*H*-1,2,3-triazol-4-yl)-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2*H*)-one (**44**): (azidomethyl)cyclopropane was freshly prepared by refluxing (bromomethyl)cyclopropane (0.49 g, 3.6 mmol) and sodium azide (0.47 g, 7.2 mmol) in 4 mL MeOH and 0.5 mL  $H_2O$  for 16 h. To a tube 1 mL of the azide solution (0.8 M) was added followed by alkyne **43** (50 mg, 0.15 mmol), (+)-sodium L-ascorbate (3 mg, 0.015 mmol) and  $CuSO_4 \cdot 5 H_2O$  (4 mg, 0.015 mmol), after which the tube was sealed. The reaction was stirred overnight at room temperature after which the reaction was quenched in 1 M aqueous  $Na_2CO_3$  (25 mL) and extracted with EtOAc ( $2 \times 25$  mL). The organic layer was washed with 1 M aqueous  $Na_2CO_3$  ( $2 \times 25$  mL) and brine (25 mL), dried over  $Na_2SO_4$  and filtered off. The remaining residue was concentrated *in vacuo* and purified over  $SiO_2$  using a gradient of 70% heptanes in EtOAc toward 30% heptanes in EtOAc yielding 27 mg (0.06 mmol, 43%) of the title compound as a white solid.  $^1H$ -NMR: (500 MHz,  $CDCl_3$ )  $\delta$  8.66 (d,  $J=2.3$  Hz, 1H), 8.13 (s, 1H), 7.92 (dd,  $J=8.8, 2.4$  Hz, 1H), 7.03 (d,  $J=8.8$  Hz, 1H), 5.06 (hept,  $J=6.7$  Hz, 1H), 4.28 (d,  $J=7.2$  Hz, 2H), 3.99 (s, 3H), 3.28–3.18 (m, 1H), 2.70–2.64 (m, 1H), 2.59–2.50 (m, 1H), 1.81–1.57 (m, 5H), 1.49–1.33 (m, 5H), 1.32 (d,  $J=6.6$  Hz, 3H), 1.25 (d,  $J=6.7$  Hz, 3H), 0.75–0.68 (m, 2H), 0.52–0.44 (m, 2H).  $^{13}C$ -NMR: (126 MHz,  $CDCl_3$ )  $\delta$  166.70, 156.47, 152.97, 142.69, 138.93, 133.38, 128.32, 126.80, 125.27, 122.79, 121.77, 119.38, 111.22, 55.70, 54.95, 46.34, 36.80, 35.38, 25.64, 24.27, 24.01, 22.07, 20.66, 20.21, 11.31, 4.24. LC–MS (ESI)  $m/z$  found: 422  $[M+H]^+$ ; retention time: 5.12 minutes. HRMS-ESI  $[M+H]^+$  calculated for  $C_{24}H_{31}N_5O_2$ : 422.2551, found 422.2543.

## Author Contributions

M.S. and E.d.H. contributed to the synthesis of the molecules, A.M., G.C. and L.M. were involved with the parasitological screenings, M.S., L.M., G.S. and R.L. were involved in the design of the molecules and experiments. L.M., G.C., G.S., I.J.P.d.E., and R.L. obtained the necessary funding. M.S., G.S., and R.L. contributed to the writing of this manuscript. All authors have read and checked the manuscript.

## Abbreviations

DME dimethoxyethane  
DMF *N,N*-dimethylformamide  
Dppf 1,1'-ferrocenediyl-bis(diphenylphosphine)  
ESI electrospray ionization  
MTBE methyl *tert*-butyl ether  
VL visceral leishmaniasis

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## Conflict of Interest

The authors declare no conflict of interest.

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